SHORT REPORT

Newly acquired kiwi fruit allergy after bone marrow transplantation from a kiwi-allergic donor

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Abstract

Background The phenomenon of allergy transfer from an allergic donor to a non-allergic recipient via hematopoietic cell transplantation has been described by several reports. However, it could not yet been conclusively shown that allergic reaction of the recipient is elicited by the donor's cells.

Objectives In the case of a 46-year-old male patient who – for the first time in his life – had two episodes of oral allergic syndrome upon kiwi consumption after having received myeloablative hematopoietic stem cell transplantation (HCT) from his kiwi-allergic sister, we aimed to clarify the origin of allergen reactive cells in the donor. We not only intended to demonstrate if allergy was transferred by HCT but also to present an experimental workup for the analysis of allergy transfer by HCT.

Methods Allergic sensitization to kiwi in recipient and donor was proven by ImmunoCAP. Furthermore, origin of peripheral blood mononuclear cells (PBMCs) was analyzed by chromosomal fluorescence *in situ* hybridization (FISH). To confirm allergic reaction and activation of hematopoietic cells by customized kiwi extract, we performed basophil activation test from whole blood as well as T cell proliferation assays from purified PBMCs of both recipient and donor.

Results Basophil activation upon kiwi extract was demonstrated in both recipient and donor. Besides, we showed proliferation of $CD4^+$ T cells after incubation with kiwi extract. FISH analysis proved that hematopoietic cells of the male recipient completely originated from the female donor.

Conclusion Exemplified in this patient, we show for the first time that allergy transfer is mediated by the donor's cells. Moreover, our experimental approach using customized kiwi extract to prove contribution of kiwi-specific T and B cells in both kiwi-allergic recipient and donor could serve as a model approach for future studies.

Received: 18 November 2015; Accepted: 23 December 2015

Conflicts of interest

The authors declare no conflict of interest.

Funding source

This work was supported by the German Research Foundation (EY97/3-1, TR22, SPP1395/InKoMBio Busch 900/6-1), the Helmholtz Association ("Impuls- und Vernetzungsfonds"), the Bavarian Academy of Sciences (Young scholar program), CK-CARE (Christine Kühne Center for Allergy Research and Education), and FONDATION ACTERIA.

Introduction

Transfer of allergic diseases after allogeneic hematopoietic cell transplantation (HCT) is described by several reports of patients who developed allergic diseases after HCT.[1–3] However, a final proof of direct transfer of allergy has not been given so far.

Here, we present a case of allergy transfer following HCT and show for the first time that allergy is transferred and elicited by the donor's cells. Moreover, we propose a feasible experimental approach to investigate allergy transfer via HCT.

Report

The 46-year-old male recipient received myeloablative HCT from his kiwi-allergic sister at the age of 26 because of acute

†Equally contributed to this work.

(a)

Characteristic	Donor	Recipient
Sex	F	M
Age (in years)	55	46
Onset of kiwi allergy	Since earlychildhood	First episode with oral allergic syndrome 3 weeks afte rHCT
Allergen sensitizations detected by ImmunoCAP (negative: <0.1KU/l)	*D. pteronyssinus* (0.96 KU/l, class II) *D. farinae* (1.28 KU/l, class II) Crab (0.46 KU/l, class I) Carot (0.55 KU/l, class I) Kiwi ((4.81 KU/l, class III)	Kiwi (2.25 KU/l, class II)

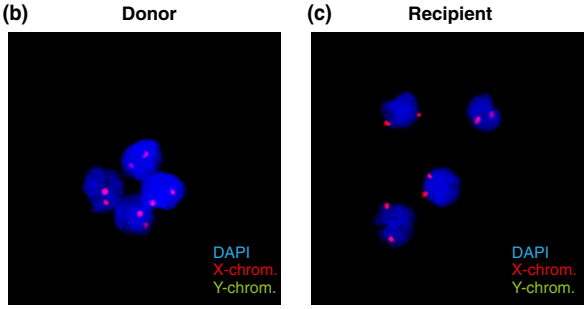

Figure 1 Characteristics of kiwi-allergic HCT donor and kiwi-allergic recipient. (a) ImmunoCAP analysis confirmed kiwi sensitization in both, donor and recipient. (b) and (c) FISH analysis of PBMCs of donor and recipient showed two signals for the X-chromosomes (b = recipient, c = donor) but no signal for the Y-chromosome confirming complete chimerism after allogeneic HCT.

lymphocytic leukaemia. Three weeks after HCT and again several months thereafter he experienced, for the first time in his life, two episodes of oral allergic syndrome upon kiwi consumption. Both, recipient and donor, reported itching and burning sensation of lips and mouth as well as oral swelling upon kiwi consumption and thus maintained dietary exclusion.

We first confirmed allergic sensitization to kiwi by routine serum ImmunoCAP (Thermo Fisher Scientific, Waltham, MA, USA)[4] analysis of both HCT recipient and donor which showed significantly upregulated IgE level for kiwi allergen (Fig. 1a). To rule out the possibility that kiwi allergy was caused by residual cells of the recipient, chromosomal FISH (Abbott, Wiesbaden, Germany) was performed with probes for the centromeric region of the X-chromosome and the heterochromatic region on the long arm of the Y-chromosome respectively (Fig. 1b,c). All PBMCs of the male recipient showed two signals for the X-chromosome and none for the Y-chromosome confirming that hematopoietic cells of the recipient completely originated from the female donor.

To gain an extended insight into specific immune cell responses causing the kiwi allergy beyond routine diagnostics, further cell culture experiments were designed. Like Mempel *et al.* who have successfully desensitized a patient with severe kiwi allergy by customized sublingual swallow allergen immunotherapy, we prepared kiwi extract by homogenization of fresh kiwi fruit pulp.[5] We then cleared the lysate by a centrifugation step and adjusted pH to 7.0. The kiwi extract was then sterile filtrated and heat inactivated at 90 °C for 10 min to inactivate potentially harmful proteases (Fig. S1a). Protein concentration as measured by BCA (Thermo Fisher Scientific) was 36 mg/mL. Before using in cell culture experiments, the extract was titrated on PBMCs to define non-toxic concentrations by Live/Dead staining. 1.44 mg/mL of the kiwi extract was defined as non-toxic concentration (Fig. S1b,c) and used in all following assays.

In a first step, Kiwi extract was used for basophil activation test (BAT, Bühlmann, Schönenbuch, Switzerland) with blood of both, HCT recipient and donor as well as with blood of a non-kiwi-allergic control (Fig. 2a,b). Robust basophil activation was observed in recipient and donor (32.3% and 77.3% activated basophils respectively), which is contrasted by absent basophil activation in the non-allergic control (0.63% activated basophils) confirming kiwi-specific effect. To detect IgE independent contribution to kiwi allergy, we designed two different proliferation assays. Radioactive labelled Thymidine (^{3}H) incorporation of PBMCs after treatment with Kiwi extract showed increased proliferation of cells from the recipient and the donor (6.9 and 3.4 fold respectively), whereas no proliferation could be detected for the non-allergic control (Fig. 2c). We next investigated CFSE-labelled PBMCs incubated with kiwi extract for 7 days. FACS analysis was used to determine kiwi-specific proliferation and to further characterize proliferating cells on single-cell level (Fig. 2d,e). Increased percentage of proliferating $CD3^+$ T cells (CSFE low) ($CFSE^{low}CD3^+$) from the recipient as well as from the donor after treatment with kiwi extract (3.3% and 9.7% respectively, Fig. 2d) was consistent with the data from the Thymidine incorporation assay. Moreover, we found that the proliferating T cell population was mainly CD4 positive (94.2% for the recipient and 89.5% for the donor, Fig. 2e). Additionally, a high percentage of T cells were positive for CD86 underlining their memory phenotype[6] (83.3% for the recipient and 77.3% for the donor, Fig. 2e).

Discussion

The hypothesis that allergies may be transferred via HCT has been proposed for long time.[1] As food allergies and, in particular, kiwi allergy are less common than, for example, pollen allergy[7,8] the argument of spontaneous acquisition of allergy seems unlikely in our patient. Moreover, we could clearly rule out this possibility in our HCT recipient: Besides many reported cases of allergy transfer by HCT, we could prove, for the first

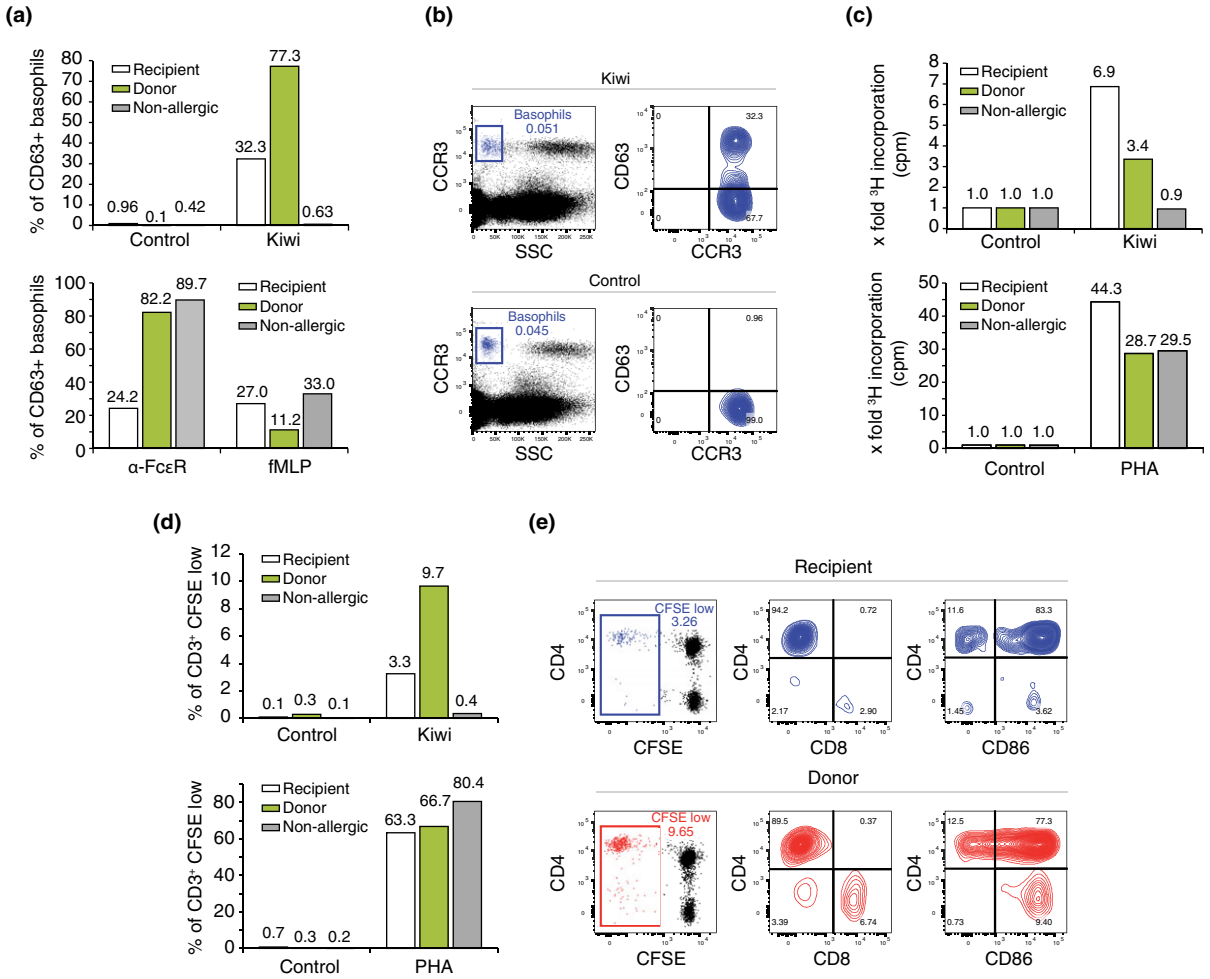

Figure 2 Immune cell response of kiwi-allergic HCT donor and kiwi-allergic recipient. (a) BAT revealed increased basophil activation ($CCR3^+CD63^+$) for the recipient (white bar) as well as for the donor (green bar) upon stimulation with kiwi extract compared to unstimulated controls (control) and kiwi stimulation of cells from a non-allergic control (grey bar). α-FcεR and fMLP served as positive controls and showed proper basophil reactivity of recipient, donor and non-allergic donor. (b) Representative FACS plots of BAT from the recipient upon kiwi stimulation (upper row) and background control (lower row) show gating strategy on activated basophils. (c) Proliferation of PBMCs of the recipient as well as of the donor was observed upon kiwi stimulation after 7 days compared to unstimulated controls (control) and kiwi-stimulated cells from a non-allergic control as measured by radioactive labelled thymidine (^3H) incorporation. PHA served as a positive control and showed proper proliferation capacity of PBMCs from the recipient, the donor as well as from non-allergic control. (d) Monitoring of CFSE-labelled PBMCs after 7 days by FACS analysis showed significant proliferation of kiwi-stimulated $CD3^+$ T cells ($CD3^+CFSE^{low}$) from the recipient as well as from the donor compared to unstimulated cells (control) and kiwi-stimulated cells from a non-allergic control. PHA as a positive control showed proper proliferation capacity of $CD3^+$ T cells from the recipient, the donor as well as from the non-allergic control. (e) Further staining of proliferating $CD3^+$ T cells upon kiwi stimulation for CD4, CD8 and CD86 showed a predominance of the $CD4^+CD86^+$ phenotype.

time, that allergy was definitely elicited by the donor's cells as the recipient showed complete chimerism and therefore kiwi-reactive cells in the HCT recipient were fully derived from the donor.

Transfer of IgE has been assumed to be responsible for allergic reactions in the recipient; however, this mechanism is irrelevant in our recipient. As IgE was shown to have a short half-life,[9] transferred donor's IgE cannot elicit allergic reactions several months after HCT and can even less explain elevated IgE levels for Kiwi allergen 20 years after HCT as in our recipient.[1] In contrast, our data clearly show a contribution of kiwi-specific T and B cells including a high percentage of memory T cells that are derived from the donor.

Kiwi allergy has been shown to be reliably detectable via prick-to-prick tests with fresh kiwi fruits. In contrast, commercially available ImmunoCAP tests and skin prick test extracts for

kiwi had reached significantly lower sensitivities[10] and are not compatible for use in cell culture experiments. Here, we showed that converting kiwi fruit into an extract applicable for cell culture experiments is possible. Besides, we confirm previous findings that heat processed kiwi fruit can still induce IgE reactivity[11] and moreover, can stimulate T cells.

Our case is unique in showing clearly donor T and B cells contribution over simple IgE transmission in long-lasting allergy transfer after HCT. Besides, rather allergen-specific proliferating Th cells than proliferating B cells seem to play a role in the pathogenesis of allergy as shown by Ueno-Yamanouchi *et al.*, which is also underlined by our data showing $CD3^{+}CD4^{+}$ T cells as kiwi-specific proliferating cell population with a memory phenotype.[12]

Allergy transfer via HCT is a rare phenomenon though insights into mechanisms would certainly extend knowledge concerning the pathophysiology of allergy. Thus, detailed experimental work-up of every single case of allergy transfer is required. In our case, we were only able to experimentally characterize the status of kiwi allergy in recipient and donor after HCT. However, with this case we propose a model work-up testing specific allergies on multiple cellular levels by customized extracts that could be used for prospective cohort analyses characterizing donors and recipients for specific allergies in detail before and after HCT.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Fig. S1 Generation and Toxicity Test of Kiwi extract.